

Outbreak of dengue virus serotype-2 (DENV-2) of Cambodian origin in Manipur, India - Association with meteorological factors

T. Sankari, S.L. Hoti, Th Bhubonchandra Singh* & J. Shanmugavel

*Microbiology & Molecular Biology Division, Vector Control Research Centre (ICMR), Puducherry & *State Epidemiologist/State Surveillance Office (SSO), Integrated Disease Surveillance Programme (IDSP), Manipur, India*

Received October 18, 2010

Background & objectives: Dengue is a major health problem in many parts of India and its neighbouring countries. Dengue cases have not been reported from Manipur, a northeastern State of India till 2007. But, the sudden outbreak of fever with febrile illness during 2007 and 2008, suspected to be dengue/dengue haemorrhagic fever was investigated to detect the causative agent. Potential impact of climatic variables on dengue transmission has been documented and hence the association between climatic factors, entomological parameters and dengue cases was also analysed.

Methods: Forty two and 16 blood samples were collected from patients suspected to have dengue infection in the year 2007 and 2008, respectively. Viral RNA was extracted from serum samples and subjected to multiplex one step RT-PCR assay. Dengue specific amplicons were sequenced and phylogenetic analysis was carried out. Multiyear trend analysis and 't' test were performed for the comparison of different meteorological variables between the years 2000-2004 and 2005-2008.

Results: The aetiological agent was found to be DENV-2 and the phylogenetic analysis showed that the isolate was similar to that of Cambodian isolate. There was a significant difference in minimum temperature ($P<0.05$), Relative humidity - morning hours ($P<0.001$), relative humidity - afternoon hours ($P<0.01$) and cumulative precipitation ($P<0.05$) between the years 2000-2004 and 2005-2008.

Interpretation & conclusions: The sudden outbreak of dengue fever in Manipur State occurred was possibly due to the increased temperature, relative humidity and decrease in cumulative precipitation. These climatic factors would have contributed to the *Aedes* mosquito abundance and increased virus transmission. Proper diseases surveillance system integrated with meteorological warning system and management of vector breeding sites will prevent such outbreaks in future.

Key words Climatic variables - dengue cases - dengue outbreak - DENV-2 - India - Manipur

Dengue has been the most significant vector borne disease with increasing distribution and incidence of cases in the recent decades. An estimated 2.5 billion people in the world are at risk of dengue fever (DF) and dengue haemorrhagic fever (DHF), of whom 1.3 billion people live in South East Asian Countries¹. Ten of eleven countries in South East Asia region including India are endemic for dengue disease, where all the four serotypes of the virus have been circulating. Dengue has become a major public health problem in many parts of India with dramatic increase in cases almost every successive year. However, dengue is hypoendemic in North Eastern States of India despite the fact that it is surrounded by several dengue endemic countries, (Myanmar, Thailand, Indonesia, *etc.*) that have been experiencing frequent major outbreaks with several deaths². So far, there is only one report of serological evidence indicating the occurrence of dengue virus serotype -2 (DENV-2) activity in Assam and Nagaland States of North East India in the early nineties³. Dengue has not been reported in Manipur, a northeastern State of India, till 2007, in spite of the presence of risk factors such as heavy precipitation and dense forest vegetation that favour dengue vector breeding⁴. However, Manipur State experienced the first sudden outbreak of fever with febrile illness from late 2007 through middle of 2008. Around 275 clinically suspected cases of dengue were reported from the local hospital of Moreh town of Chandel district, Manipur. An entomological survey in Manipur during 2004-2005 revealed that there was an increase in mosquito fauna, including potential vectors of dengue and Japanese encephalitis in Manipur⁵. The most decisive factor that determines the dynamics of dengue epidemics is the mosquito vector population.

Aedes mosquito survival and fecundity are greatly influenced by the climatic factors. It has been reported that meteorological variables like temperature have profound impact on the dengue virus transmission, population size and survival of *Aedes* mosquitoes^{6,7}. Changes in climatic variables not only influence the vector survival but also have a profound impact on rate of dengue virus transmission^{7,8}. Many studies have documented the association between the meteorological variables and dengue epidemics including the Asian countries like China and Singapore¹⁰⁻¹². The mere presence of vector alone may not result in an epidemic in the absence of viral source. The main precipitating factors of dengue epidemics are introduction of virus from an endemic to non-endemic area through human migration and abundant vector population influenced

by the climatic variables. Hence the purpose of this study was to investigate the suspected dengue outbreak in Moreh town of Manipur and identify the aetiological agent, and to analyse the influence of meteorological factors on the dengue epidemic that occurred for the first time in Manipur State of India.

Material & Methods

Study area: Moreh, an international trade centre town of Chandel district in Manipur State, India, is located between 94° 15' E longitude and 24° 10' N latitude, bordering Myanmar which is 5 km away from Tamu, the counterpart of Myanmar. This region is unique in temporal and spatial characteristics when compared to those of other northern, southern or western parts of India and is situated at a height of 790 meters above the sea level. The estimated population of Chandel district of Manipur in 2007 was 26,19,429 covering an area of 3,313 sq.km¹³. The average daily minimum and maximum temperature in Manipur ranges from 15 to 32°C. However, the daily minimum temperature reaches to 0° C in winter and the maximum temperature rises to 32° C during summer. Manipur State receives an average rainfall of 1467.5 mm, annually.

Data collection: Meteorological data registered daily for the period 2000 through 2008 including maximum and minimum temperature, relative humidity and cumulative precipitation were obtained from Indian Agricultural Research Institute, Manipur. Extreme weather events for the above period and the annual cumulative precipitation were compiled from Indian Meteorological Division and India Statistics website^{14,15}. The office of the State surveillance unit, Manipur, provided the entomological parameters collected during the epidemic.

Blood collection and serotype identification: A rapid response team from Manipur State surveillance unit visited Moreh town for investigation during the later part of September 2007 following an outbreak of febrile illness and reported that there were around 275 suspected dengue cases with fever and two deaths. Forty two blood samples (1 ml) from patients with suspected dengue symptoms (as per WHO guidelines) in October- December 2007 collected during the last quarter of 2007 and another 16 samples from second quarter of 2008 were transported after separation of serum separately on dry ice to Vector Control Research Centre (VCRC), Puducherry for serological diagnosis of dengue and serotype identification.

RNA extraction and reverse transcription PCR: Viral RNA was extracted from acute phase serum samples using QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and subjected to multiplex reverse transcription polymerase chain reaction (RT-PCR) following the procedure of Harris *et al*¹⁶ with minor modifications in the PCR conditions using One-Step RT-PCR Kit (Qiagen, Hilden, Germany). DNA amplified was analyzed by 2 per cent agarose gel electrophoresis.

Nucleotide sequencing and phylogenetic analysis: DNA from the gel was extracted, purified and sequenced using Big Dye terminator V3.1 Ready Reaction Sequencing Mixture and analyzed in an automated 3130 × 1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences obtained were subjected to similarity search using BLAST server of National Centre for Biotechnology Information GenBank database release 143.0 (NCBI, National Institutes of Health, Bethesda, MD, USA). Blast selected sequences were retrieved from GenBank and aligned using ClustalW¹⁷. Phylogenetic tree was constructed by Neighbor-joining method (1000 bootstrap replicates) using Mega software, version 4.0¹⁸.

Anti-dengue IgM antibody detection: Anti-dengue immunoglobulin (IgM) capture ELISA was also performed on the suspected samples to detect circulating dengue antibodies using PanBIO diagnostics kit (Pan Bio Diagnostics, Australia) as per manufacturer's instructions.

Analysis of meteorological variables and dengue cases: Monthly mean maximum and minimum temperatures were calculated from the daily mean values from 2000 through 2008. Relative humidity (RH) is an indicator of likelihood of precipitation and the daily relative humidity recorded in the morning and afternoon were compiled and the average monthly RH and the monthly cumulative precipitation were calculated. Multiyear trend analysis of climatic factors was performed for the years 2000 through 2008. Since there was an increase in mosquito density during the year 2005, 't' test was performed to compare the meteorological variables, dengue cases and Breteau Index (BI) during 2000-2004 with that of climatic anomalies, dengue cases and BI of 2005-2008.

Results

The epidemic started in September 2007 with the appearance of suspected cases and reached a peak by December 2007, followed by another peak in June 2008.

The minimum and maximum ages of the suspected cases were 7 and 77 yr, respectively. Clinically, there were only two DHF cases, while the remaining ones were identified as DF cases.

Antibody detection and serotyping: Of the 42 and 16 samples tested for IgM anti-dengue antibodies, 25 and 2 were positive, respectively. Twenty (47%) of 42 samples from 2007 batch and 12 (75%) of 16 from 2008 batch were randomly selected and subjected to RT-PCR to determine the serotype of dengue virus. Seventeen of the 20 samples collected in 2007 and 10 of the 12 samples from 2008 tested were positive for dengue viral RNA by RT-PCR assay and the diagnostic band size of 119 bps, indicative of DENV-2 was detected.

Sequence similarity and submission: The nucleotide sequences of the dengue virus obtained from Manipur were found to be 100 per cent similar to that of DENV-2 of Cambodian origin upon Blast search and multiple alignment. The sequences were submitted to GenBank nucleotide database (FJ769285 and FJ769286). The phylogenetic tree constructed also showed resemblance of Manipur isolate to 2007 isolate of Cambodian origin (Fig. 1).

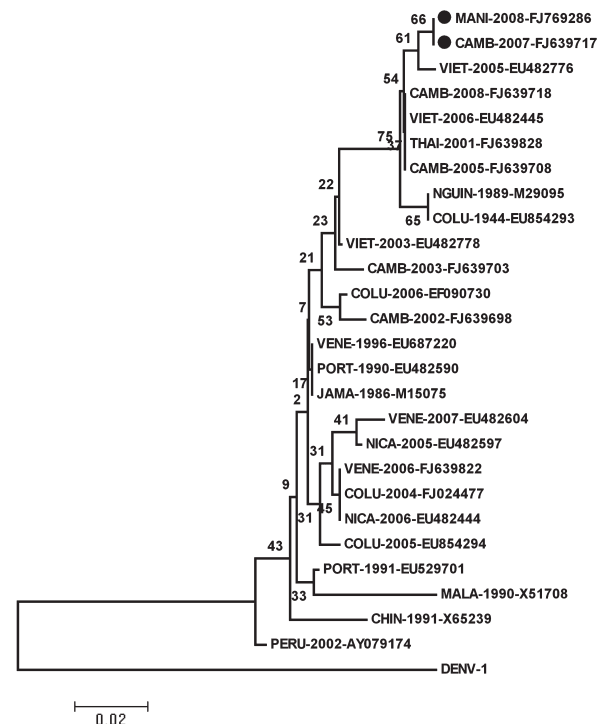


Fig. 1. Phylogenetic relationship of Capsid gene segment of DENV-2 isolate from Manipur and reference DENV-2 sequences from GenBank. Virus isolates from Manipur grouped with Cambodian isolate 2007 are shown with markers. The scale bar indicates the genetic distance. DENV-1 was used as the outgroup virus.

Multiyear trend analysis of climatic factors: Multiyear trend analysis of meteorological factors (Fig. 2) showed that there was a significant increase in mean minimum temperature during 2005-2008 ($P<0.034$). On the contrary, there was a drastic reduction in the monthly cumulative precipitation during 2005-2008. The highest monthly maximum temperature of 34°C was recorded in the month of July 2006, followed by 33.9°C and 33.8°C in July

2007 and 2008, respectively¹⁴. Extreme low monthly cumulative precipitation of 90.3 mm was recorded during the heavy rainfall season of 2006. The overall monthly cumulative precipitation during 2000-2004 ranged from 0-490 mm with an average of 132.2 mm whereas it was 0-233.6 mm with an average of 104.3 mm for 2005-2008 ($P<0.05$). Relative humidity recorded was increased and decreased significantly in the morning and afternoon hours respectively during 2005-2008 when compared to 2000-2004. Relative humidity in the morning increased by 10 per cent in 2000-2004 when compared to 2005-2008 ($P<0.001$). Also the relative humidity in the afternoon decreased significantly during the years 2005-2008 ($P<0.05$) (Table). The house index and Breteau index for *Aedes aegypti* vector recorded during the epidemic were 25 and 56 per cent, respectively. *Ae. aegypti* mosquito house index (HI) and container index (CI) were found to be scrupulously increased during 2005⁵ and the HI and CI was >25 during the epidemic period. Comparison of meteorological variables during 2000-2004 with that of 2005-2008 showed that significant change in rainfall, minimum temperature and relative humidity could have been the reason for the increase in mosquito density and incidence of dengue cases in Manipur (Fig. 3).

Discussion

There have been reports of dengue outbreaks in most of the parts of India except, the extreme northern and northeastern States, which could be due to their unique temporal and spatial characteristics. Manipur, the only northeastern State, which was reported to be free from dengue till 2007, experienced a suspected

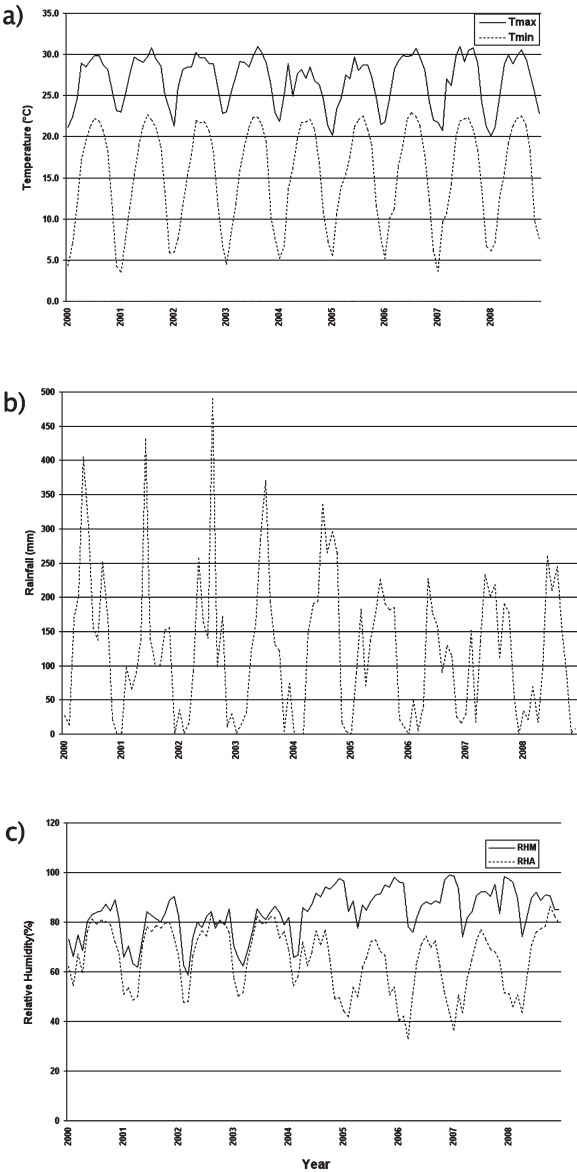


Fig. 2. Multiyear trend analysis of temperature (°C), Rainfall (mm) and Relative humidity (%). T_{max}, temperature maximum, T_{min}, temperature minimum, RHM, relative humidity morning hours, RHA, relative humidity afternoon hours.

Table. Comparison of climatic variables in relation to the dengue cases and Breteau index

| Weather variables | 2000-2004 | 2005-2008 |
|---|--------------|----------------|
| Rainfall (mm) | 134.2 ± 13.9 | 108.8 ± 19.12* |
| Relative humidity (%) (Morning hours) | 79.4 ± 3.92 | 89.1 ± 0.71* |
| Relative humidity (%) (Afternoon hours) | 69.2 ± 3.06 | 60.5 ± 4.44* |
| Temperature maximum (°C) | 27.0 ± 0.72 | 27.3 ± 0.27 |
| Temperature minimum (°C) | 15.2 ± 0.14 | 15.5 ± 0.18* |
| Dengue cases (%) | - | 67.25 |
| Breteau index (%) | - | 44.5 |

* $P<0.05$ compared to 2000-2004

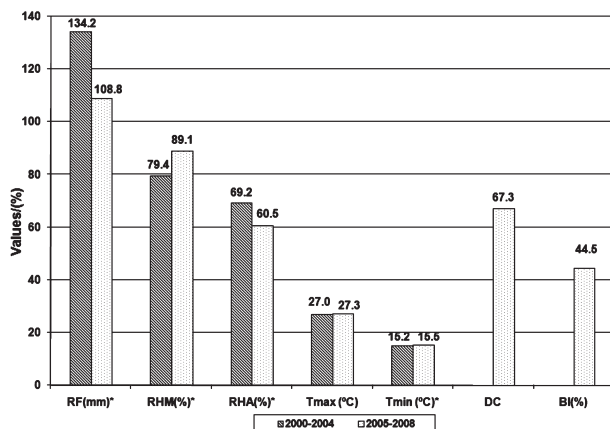


Fig. 3. Comparison of meteorological variables, dengue cases and Breteau Index between the years 2000-2004 and 2005-2008.

RF, Rainfall; T_{max} , temperature maximum; T_{min} , temperature minimum; RHM, relative humidity morning hours; RHA, relative humidity afternoon hours; DC, dengue cases; BI, breteau index.

* $P < 0.05$.

outbreak of dengue during 2007-2008. Investigation of suspected samples confirmed that the outbreak was due to dengue virus and the aetiological agent was dengue virus serotype -2. The outbreak during 2007-2008 may be the result of many confounding factors including dengue vector mosquito abundance, their activity and behaviour, weather variables like temperature, humidity, precipitation, human activities and movement of people, viral source and their serotypes. Though systematic entomologic surveys were not conducted in the State, the data collected during monsoon and post-monsoon seasons of 2005 revealed the existence of abundant mosquito population, including dengue vectors⁵. The increase in vector density itself was an indication of imminent outbreak of dengue in the State. The house index and Breteau index for *Aedes* vector recorded during the epidemic also showed that the area was at high risk of dengue transmission.

The current results showed a very significant increase (morning hours) and decrease (afternoon hours) in relative humidity, increase in minimum temperature and decrease in precipitation during 2005-2008. Thus the changes in climatic variables were positively associated with the increase in *Aedes* mosquito vector population from 2005 and also the transmission of dengue virus in 2007. Relative humidity is a crucial factor for the newly laid eggs as well the adult mosquitoes throughout the life cycle. Higher moisture facilitates better survival and emergence of larvae whereas lower humidity is

probably a limiting factor for dengue transmission as the eggs are subjected to desiccation¹⁹. There was a change in the relative humidity levels in the morning as well as in the afternoon during 2005-2008. There was a significant increase in average morning hours RH levels during 2005-2008 when compared to the RH levels (77.7%) of previous years 2000-2004, which would have prevented the excessive desiccation of eggs and successful emergence of larvae from the eggs. Hence, relative humidity was a precipitating factor that could have played a vital role in the abundant multiplication and survival of mosquitoes in Manipur State.

Another significant meteorological factor was the change in the cumulative precipitation in the State from 2005 onwards. There has been drastic reduction in precipitation from 2005 and the monthly cumulative precipitation recorded was 460 mm during 2000-2004, whereas and it was 233 mm during 2005-2008. Generally heavy precipitation in high altitude regions like Manipur eliminates breeding habitats of larval and pupal population and also reduces the survival rate of female mosquitoes in short time. Conversely, low precipitation increases the ambient temperature that increases the survival rate of mosquitoes and creates conducive environment for vector breeding through the usage of water storage containers, air coolers in the urban areas and water retention in leaf axils of banana and pine apple plantation in the rural areas²⁰.

Though the multiyear trend analysis of temperature showed little variation in maximum temperature, the highest temperature of 34°C was recorded in Manipur in 2006 and 2007¹⁴. Further, a significant increase in minimum temperature was recorded during 2005-2008 than during 2000-2004. This increase in temperature would have resulted in swift multiplication of *Aedes* mosquitoes as it experiences shortened reproductive cycle and an increased feeding frequency of more than two folds at higher temperature as compared to the lower temperature²¹. Pupal development period also reduced from four days to less than one day at a temperature range of 32 to 34°C⁸. As a result the extrinsic incubation period of dengue viruses is shortened in mosquitoes and thus increasing the rate of transmission of virus from the infected host to the uninfected host²². Similar results have been reported on temperature and its influence on relative humidity affecting the vector survival indirectly²³. Further, area in which pine apple and rubber plantations has been growing increased from 450 and 1558 hectares in 2002 to 1960 and 1859 in 2009 respectively^{24,25}. Unplanned urbanization and urban human ecology play as key factors in the adaptability of

Ae. aegypti to urban environment than *Ae. Albopictus*, a primary vector of rural areas²⁶. This was corroborated by the abundance of *Ae. aegypti* vector population in the thriving urban environment of Manipur.

In addition to the vector abundance and mosquito-genic environment, movement of human population from dengue endemic areas to non-endemic area appears to have contributed significantly to the outbreak of dengue in Manipur. Moreh is an international trade town of Manipur bordering Myanmar. Myanmar witnessed the largest dengue outbreak during 2001 and also has been experiencing frequent epidemics with all the four dengue serotypes²⁷. During 2007-2008, there was also an outbreak of dengue fever in other neighbouring countries viz., Cambodia Vietnam and Thailand²⁸⁻³⁰. Border between Manipur and Myanmar is porous and hence facilitates a relatively free migration of human population from dengue endemic countries, which could have been the primary means of virus introduction into the Manipur State. This is evidenced from the 100 per cent nucleotide similarity of the virus to the Cambodian strain. Hence, temporally, geographically and etiologically the outbreak appears to be related to Cambodian outbreak.

The dengue outbreak in 2007 and subsequent increase in the number of dengue cases highlight that this non-endemic State was changed to a hyperendemic State leaving the entire north eastern States susceptible to dengue disease. The conditions prevailing in Manipur might put this State as well as the neighbouring areas at the risk of severe form of dengue endemicity. Moreover, the preponderance of infections with DENV-3 in the adjacent country, Bangladesh as well as in India and the currently circulating DENV-2 in this State may result in multiple co-infections leading to more severe clinical forms of DHF/dengue shock syndrome (DSS). Therefore, there is a need to establish an appropriate dengue disease surveillance system to integrate it with mosquito surveillance system and meteorological early warning system. This integrated surveillance system will predict the outbreaks, and help in management of vector breeding sites, prevention of man and vector contact. Such activities conducted at least during the monsoon and post-monsoon seasons will prevent the major outbreak of dengue fever and minimize the harmful impact of outbreaks in coming years in the northeastern States of India.

Acknowledgment

Authors thank Dr M. Kalyanasundaram, Officer in-charge, Vector Control Research Centre, Puducherry, India, during 2007-2008 for his support in carrying out this work. Authors also acknowledge Dr P. Vanamail for his valuable statistical suggestions.

References

1. *Dengue status in South East Asia region: An epidemiological Perspective* SEA/RC61/R5. Country Dengue Morbidity/Mortality Statistics Report; 2008.
2. World Health Organization. Regional Office for South-East Asia. World Health Organization. Regional Office for South-East Asia Dengue case fatality rates. Available from: http://www.searo.who.int/LinkFiles/Dengue_CFR_Dengue_85-06.pdf, accessed on October 2, 2010.
3. Barua HC, Mahanta J. Serological evidence of DEN-2 activity in Assam and Nagaland. *J Commun Dis* 1996; 28 : 56-8.
4. National Vector Borne Disease Control Programme. India. Dengue cases and deaths. Available from: <http://nvbdcp.gov.in/dengueall.html>, accessed on October 8, 2010.
5. Dutta P, Khan SA, Khan AM, Sharma CK, Mahanta J. Biodiversity of mosquitoes in Manipur State and their medical significance. *J Environ Biol* 2005; 26 : 531-8.
6. Yang HM, Macoris MLG, Galvani KC, Andrighetti MTM, Wanderley DMV. Assessing the effects of temperature on the population of *Aedes aegypti*, the vector of dengue. *Epidemiol Infect* 2009; 137 : 1188-202.
7. Focks DA, Brenner RJ, Hayes J, Daniels E. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *Am J Trop Med Hyg* 2000; 62 : 11-8.
8. Watts DM, Burke DS, Harrison BA, Whitmire RE, Nisalak A. Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. *Am J Trop Med Hyg* 1987; 36 : 143-52.
9. Fouque F, Carinci R, Gaborit P, Issaly J, Bicout DJ, Sabatier P. *Aedes aegypti* survival and dengue transmission patterns in French Guiana. *J Vector Ecol* 2006; 31 : 390-9.
10. Lu L, Lin H, Tian L, Yang W, Sun J, Liu Q. Time series analysis of dengue fever and weather in Guangzhou, China. *BMC Public Health* 2009; 9 : 395.
11. Hii YL, Rocklöv J, Ng N, Tang CS, Pang FY, Sauerborn R. Climate variability and increase in intensity and magnitude of dengue incidence in Singapore. *Glob Health Action* 2009; 2 .
12. Wu PC, Guo HR, Lung SC, Lin CY, Su HJ. Weather as an effective predictor of occurrence of dengue fever in Taiwan. *Acta Trop* 2007; 103 : 50-7.
13. Directorate of Economics & Statistics. *Economic survey Manipur*. Imphal: Government of Manipur; 2008-2009.
14. Indian meteorological division. Available from: <http://www.imd.gov.in>, accessed on October 8, 2010.
15. Datanet India Pvt. Ltd. Ministry of Statistics and Programme Implementation: Government of India. c2000 [updated 2008]. Sub-division-wise actual and normal rainfall in India (2002 to 2007). Available from: <http://www.Indiastat.com>, accessed on December 27, 2009.

16. Harris E, Roberts TG, Smith L, Selle J, Kramer LD, Valle S, *et al*. Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase-PCR. *J Clin Microbiol* 1998; 36 : 2634-9.
17. Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinformatics* 2002; 2 : Unit 2-3.
18. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2000; 24 : 1596-9.
19. Luz C, Tai MH, Santos AH, Silva HH. Impact of moisture on survival of *Aedes aegypti* eggs and ovicidal activity of *Metarhizium anisopliae* under laboratory conditions. *Mem Inst Oswaldo Cruz* 2008; 103 : 214-5.
20. Dutta P, Prakash A, Bhattacharyya DR, Khan SA, Gogoi PR, Sharma CK, *et al*. Mosquito biodiversity of Dibru-Saikhowa biosphere reserve in Assam, India. *J Environ Biol* 2010; 31 : 695-9.
21. Tun-Lin W, Burkot TR, Kay BH. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Med Vet Entomol* 2000; 14 : 31-7.
22. Watts DM, Burke DS, Harrison BA, Whitmire RE, Nisalak A. Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. *Am J Trop Med Hyg* 1987; 36 : 143-52.
23. Thu HM, Aye KM, Thein S. The effect of temperature and humidity on dengue virus propagation in *Aedes aegypti* mosquitoes. *Southeast Asian J Trop Med Public Health* 1998; 29 : 280-4.
24. North East Resource Databank. India: Horticulture in Manipur; cNEDFi [updated 2009]. Area Under some fruits and vegetables in Manipur 2001-02 to 2007-08; [about 2 screens]. Available from: <http://db.nedfi.com/content/horticulture-manipur>, accessed on April 9, 2010.
25. North East Resource Databank. India: Rubber Plantation; cNEDFi [updated 2008 Dec 15]. State-wise Area and Production of Rubber in North Eastern States; [about 4 screens]. Available from: <http://db.nedfi.com/content/rubber-plantation-north-east-india>, accessed on April 9, 2010.
26. Bang YH, Shah NK. Human ecology related to urban mosquito-borne diseases in countries of South East Asia region. *J Commun Dis* 1988; 20 : 1-17.
27. Thu HM, Lowry K, Myint TT, Shwe TN, Han AM, Khin KK, *et al*. Myanmar dengue outbreak associated with displacement of serotypes 2, 3, and 4 by dengue 1. *Emerg Infect Dis* 2004; 10 : 593-7.
28. Kmietowicz Z. Cambodia faces dengue fever epidemic. *BMJ* 2007; 335 : 65.
29. [No authors listed]. Cases of dengue fever on rise in Vietnam. *Chin Med J (Engl)* 2008; 121 : 2464.
30. Hedddini A, Janzon R, Linde A. Increased number of dengue cases in Swedish travellers to Thailand. *Euro Surveill* 2009; 14 : 1911-2.

Reprint requests: Dr S.L. Hoti, Scientist "F", Molecular Biology & Biotechnology, Vector Control Research Centre (ICMR), Indira Nagar, Puducherry 605 006, India
e-mail: slhoti@yahoo.com